NEUROACTIVE STEROIDS PROTECT FROM BORTEZOMIB-INDUCED TOXICITY: EVIDENCE FROM *IN VITRO* MODELS

Fabbro V[1], Tonelli E[1,2], Scuteri A[1,3], D'Aprile C[1], Naghshbandieh A[1,2], Lim D[4], Distasi C[4], Giatti S[5] and Meregalli C[1]

[1]School of Medicine and Surgery, Experimental Neurology Unit and Milan Center for Neuroscience, University of Milano-Bicocca, Monza, Italy; [2]University of Milano-Bicocca, PhD Program in Neuroscience, Monza, Italy; [3]Milan Center for Neuroscience (NeuroMI); [4]University of Piemonte Orientale, Department of Pharmaceutical Sciences, Novara, Italy; [5]University of Milan, Department of Pharmacological and Biomolecular Sciences "Rodolfo Paoletti", Milan, Italy

Chemotherapy has significantly improved patient survival but has also increased the incidence of chemotherapy-induced peripheral neuropathy (CIPN), a common side effect that affects the patients' quality of life and limits treatment efficacy. Among the agents associated with CIPN development, bortezomib (BTZ) - a proteasome inhibitor used in the treatment of multiple myeloma - is known to induce painful peripheral neuropathy (BIPN), for which analgesic options remain limited. Recent evidence suggests that neuroactive steroids (NAS), cholesterol derivatives with already proven beneficial effects towards both the central and the peripheral nervous system, may promote neuroprotection in CIPN models. For our study, we selected a palette of different NAS for screening and dose-finding purposes. First, we tested them (alone and in combination with BTZ) in two different cell lines: F11, a somatic cell hybrid of a rat embryonic dorsal root ganglion (DRG) and mouse neuroblastoma cell line N18TG2, and MSC80, a mouse Schwann cell line. To assess the effects of these treatments, cell viability in F11 and MSC80 cell lines was evaluated via crystal violet assay after 24 h of treatment with BTZ (10 nM) alone or in combination with the respective NAS (100 nM). Based on the results, we selected three NAS for further experiments: pregnenolone (PREG) and dihydroprogesterone (DHP), that partially but significantly prevented BTZ-induced cytotoxicity in F11 and MSC80, respectively, as well as allopregnanolone (ALLO), that was mildly protective in both cellular models. Subsequently, we decided to test the abovementioned combinations (BTZ + PREG/DHP/ALLO) in a more complex *in vitro* model, using organotypic cultures from embryonic rat DRGs. DRGs from E15 rat embryos were treated with a toxic dose of BTZ (5 nM) for 24 or 48 h. The neurotoxic effect was assessed by measuring neurite length of DRG explants. ALLO and PREG were able to protect from the toxicity induced by BTZ exposure at different degrees in both time points, demonstrating their neuroprotective action, whereas DHP was ineffective at any time point. Taken together, our results highlight PREG and ALLO as promising neuroprotective agents in BIPN *in vitro* models, paving the way for future experiments aimed at testing their efficacy in BIPN *in vivo* models as well as addressing their specific mechanism of action in counteracting BTZ-induced toxicity.

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EX VIVO MAPPING OF NIGRO-THALAMIC DOPAMINERGIC TERMINALS REVEALS REGIONAL SPECIALIZATION AND SYNAPTIC MARKERS IN THE HUMAN THALAMUS

Cirillo G[1], Montella M[2], La Mantia E[2], Tedesco I[2], De Luca C[1], Virtuoso A[1], De Angelis F[1], Riccio L[1], Esposito F[3], Cirillo M[3], Franco R[2] and Papa M[1]

[1]Division of Human Anatomy, Neuronal Networks Morphology & Systems Biology Lab, Department of Mental and Physical Health and Preventive Medicine, University of Campania "Luigi Vanvitelli, Naples, Italy; [2]Division of Pathology, Department of Mental and Physical Health and Preventive Medicine, University of Campania "Luigi Vanvitelli, Naples, Italy; [3]Department of Advanced Medical and Surgical Sciences, Advanced MRI Research Center, University of Campania Luigi Vanvitelli, Naples, Italy

The thalamus plays a crucial role in integrating subcortical inputs and relaying them to cortical circuits, yet the extent and specificity of dopaminergic innervation to human thalamic nuclei remain incompletely understood. Recent tractography-based studies suggest the existence of direct nigro-thalamic pathways, but direct histological evidence is still limited. In this study, we provide an *ex vivo* mapping of dopaminergic projections to the human thalamus using high-resolution immunohistochemistry and confocal microscopy. Human brain specimens were processed to detect multiple dopaminergic markers, including tyrosine hydroxylase (TH), vesicular monoamine transporter 2 (VMAT2), and aromatic L-amino acid decarboxylase (AADC), across anatomically defined thalamic nuclei. Multilabel immunofluorescence and 3D reconstruction allowed precise identification of axonal arborization and synaptic varicosities within the mediodorsal (MD), and ventral anterior (VA)/ventral lateral (VL) nuclei. Quantitative analysis revealed a significant expression of TH+/VMAT2+ fibers and D2R staining in the MD/VA/VL nuclei, supporting the notion of region-specific dopaminergic input. These results provide the first immunohistological confirmation of selective dopaminergic innervation of the human thalamus. This structural evidence complements prior neuroimaging findings and suggests a potential role for the nigro- thalamic pathway in modulating thalamo-cortical circuits involved in executive and cognitive functions.